#### Systematics

# Systematics of Two Feeding Morphs of *Schizocerella pilicornis* (Hymenoptera: Argidae) and Recognition of Two Species

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ABSTRACT A number of species of Argidae were proposed in the late 1800s and early 1900s, all of which later were regarded as a single color-variable species, *Schizocerella pilicornis* (Holmgren). In the mid-1970s, an external-feeding type was reported in the literature, and it also was identified as *S. pilicornis*, although previously *S. pilicornis* was only known to feed by leaf mining. Thus, the uniqueness of a species with two distinct feeding types led us to question the taxonomy of *S. pilicornis*. We use data from the mitochondrial cytochrome oxidase I gene (COI) to show that the two feeding types have a sequence divergence of 5.8%, which exceeds the difference between many insect taxa currently classified as different species. Considering the existing evidence on morphological, behavioral, allozymic, and ecological differences, we here recognize these two feeding types as distinct species: *Schizocerella pilicornis* (Holmgren) as the leaf-mining species and *Schizocerella lineata* (Rohwer) as the external-feeding species. We provide a diagnosis for each species, and show that adults of *S. pilicornis* have a mostly black mesonotum, whereas adults of *S. lineata* have an orange-red mesonotum.

KEY WORDS phylogenetics, speciation, Schizocerella pilicornis, mitochondrial DNA, phytophagy

Analyses of mitochondrial DNA variation have proven useful in resolving the identity of and relationships among closely related species, and in some cases populations within a species. Such studies are more meaningful when other data are available for comparison, whether morphological, ecological, or allozymic. The use of mitochondrial DNA to resolve the phylogeny of insects in general and Hymenoptera specifically has been well tested (Dowton and Austin 1994, Danforth et al. 1998, Whitfield and Cameron 1998, Danforth 1999, Kambhampati et al. 2000). The cytochrome oxidase I gene (COI) is a slowly evolving protein coding gene, yet it can resolve differences between populations or closely related species (Simon et al. 1994; Cruickshank 2002; Hebert et al. 2003a, 2003b). We describe a study where an analysis of mitochondrial DNA variation, combined with existing morphological, ecological, and allozymic data helps to resolve the status of an insect species dimorphic for feeding behavior.

Schizocerella pilicornis (Holmgren) (Hymenoptera: Argidae) commonly feeds on Portulaca oleracea L. and other members of the genus Portulaca (Portulacaceae). P. oleracea is native to North America, but it

has been introduced around the world (Danin et al. 1978, Matthews et al. 1993). Larvae of S. pilicornis feed either on the exterior of the leaf by chewing the leaf margin, or they feed by leaf mining, tunneling within the leaf interior. The leaf-mining type is found throughout North America, and although we have not observed the external feeding form in our sampling in California, Florida, Massachusetts, Maryland, or Virginia, the external-feeding type also is reported in the literature to be widespread in North America. The early literature on S. pilicornis identifies only the leafmining type (Webster and Mally 1900). However, in the mid-1970s, Stan Gorske and colleagues first reported the external-feeding type (Gorske and Sell 1976). These two feeding types of S. pilicornis, external feeding and leaf mining, are currently classified as a single species (Smith 1992). When inspected under the light microscope, larvae and adults of both feeding types have been claimed to be identical, except that the external-feeding type is slightly larger than the leaf-mining type (Gorske et al. 1977). However, when separate populations of the two feeding types are established in the laboratory, eggs produced by adults that feed by leaf mining as larvae produce larvae that feed exclusively by leaf mining, and eggs produced by external-feeding adults give rise only to external-feeding larvae (Gorske and Sell 1976; C.D.H., unpublished data).

The classification of these two feeding types as conspecific is unusual given that we are unaware of any other species of phytophagous insect that is polymor-

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Table 1. Feeding behaviors of families of phytophagous Hymenoptera (worldwide)

Families that contain multiple feeding behaviors	Families that contain a single feeding behavior
Xyelidae	Pamphiliidae
Genera are either external feeding or internal feeding	All species, genera, and subfamilies are external feeding
Tenthredinidae	Cimbicidae
Subfamilies are either external feeding or internal feeding	All species, genera, and subfamilies are external feeding
Diprionidae	Siricidae
All are external feeding except for a single concealed feeding species	All woodborers
Argidae	Xiphydriidae
All are external feeding except for the leaf mining Schizocerella pilicornis	All woodborers
Pergidae	Anaxyelidae
Phylacteophaga are leaf miners of Eucalyptus and Corynophilus pumilis	A single wood-boring species
(Klug) is a leafminer of Roupala montana (Protaceae); where	Cephidae
known other species are external feeders	All species are twig and stem borers
	Megalodontesidae
	External feeding
	Blasticotomidae
	Frond and leafminers in ferns

phic for feeding type. Moreover, only rarely are multiple feeding types represented within a single genus, although multiple feeding types do occur within families (Powell et al. 1999). We do not include species that shift feeding type ontogenetically, such as members of the Bucculatricidae, Lyonetidae, Gelechiidae, and other families of Lepidoptera that mine leaves in early instars and feed externally in later instars. We follow Strong et al. (1984) and Mitter et al. (1988) in defining phytophagous insects to be those taxa in which larvae feed "on the living tissues of higher plants." Of the 13 families of Hymenoptera that are phytophagous, eight families are represented by a single feeding type (Table 1) and within the five remaining families, which includes the Argidae, most subfamilies contain a single feeding type (Schmidt and Smith 2006). All described members of the Argidae except for S. pilicornis are known to be external feeding.

Among the genera of phytophagous Hymenoptera, only a small number have multiple feeding types or larvae that exhibit different feeding habits. Several examples are *Xyela* Dalman, *Amauronematus* Konow, *Pristiphora* Latreille, and *Dolerus* Panzer. *Xyela gallicaulis* Smith forms galls on new shoots of pine, unlike its congeners that feed in staminate cones (Smith and Middlekauff 1987). *Amauronematus* includes some species that are gall makers, although most species are free feeding (Viitasaari 2002). *P. angulata* Lindquist larvae usually develop in flower buds of *Spiraea* but can be found as free feeders on leaves in later instars (Viitasaari 2002). Larvae of *Dolerus cothurnatus* Serville feed inside *Equisetum* stems during the daytime but feed externally at night (Viitasaari 2002).

In addition to the two feeding strategies of *S. pilicornis*, there are differences in allozymes (Gorske and Sell 1976), in the duration of the developmental stages, in the size of larvae (head capsule width and body length), and the depth of pupation sites in the soil (Gorske et al. 1977). These important ecological, morphological, physiological, and genetic differences between the two feeding types suggest the presence of two distinct species. We here report additional genetic

data from the COI gene to resolve the status of the two feeding types within S. pilicornis.

#### Materials and Methods

We used specimens from California, Illinois, Indiana, and Massachusetts, representing 14 populations, nine mining and five external feeding. At several of our midwestern sample locations, the two feeding types were sympatric and could be collected on the same individual host plant. We collected larvae from the field and stored them in the laboratory at  $-80^{\circ}$ C until DNA extraction. Because leaf-mining larvae of S. pilicornis move between leaves to initiate new mines, larvae found on the exterior of the host plant could represent either feeding type. To ensure accurate identification of each feeding type, we placed larvae found on the exterior of the plant on fresh undamaged foliage until they initiated a leaf mine or were observed to feed externally. We discarded larvae we did not observe to feed at all.

We extracted DNA from larval tissue by using a standard phenol/chloroform method (Spicer 1995). We used ethanol to clean the DNA, and then we centrifuged the DNA and allowed it to dry. We resuspended the resulting DNA pellet in 50  $\mu$ l of 1× TE buffer. Using primers "Ron" (5'-GGATCACTGATATAGCATTCCC-3') and "Nancy" (5'-CCCGGTAAAATTAAAATAT AAACTTC-3'), we amplified a portion of the COI gene (Simon et al. 1994), ≈365 bp. Polymerase chain reactions (PCRs) took place in a P480 thermal cycler (PerkinElmer Life and Analytical Sciences, Boston, MA) for 30 cycles of 1) denaturing at 94°C for 30 s, 2) annealing at 52°C for 30 s, and 3) extension at 72°C for 1 min after an initial denaturation at 95°C for 30 s. We cleaned amplified PCR products before sequencing by polyethylene glycol (PEG) precipitation or with QIA-Quick columns (QIAGEN, Valencia, CA).

We did all sequencing via dye terminator cycle sequencing on a Catalyst 800 Molecular Biology Lab Station, and we closely followed the protocol specified by the ABI PRISM Dye Primer Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City,

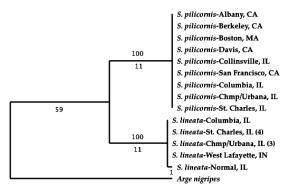


Fig. 1. Single most parsimonious tree with bootstrap support of 1,000 replicates above the branches for the 14 sampled population of *Schizocerella* sp. The values below the branches represent the number of steps between the nodes. Populations in which more than a single specimen was sequenced are noted in parentheses with the locality information.

CA). We again used primers Ron and Nancy for sequencing. We obtained sequence for the outgroup, *Arge nigripes* (Retzius) (Hymenoptera, Argidae), from GenBank, accession no. AF146680.

We aligned sequences of matching strands using Sequencher 3.0 (Gene Codes Corporation, Inc. 1995). We evaluated sequences for overall base composition bias and found no significant bias. We used the maximum parsimony algorithms of PAUP 4.0b2 (Swofford 1999) in our phylogenetic analysis, with transitions and transversions weighted equally, and assessed the degree of support for the nodes in the single most parsimonious tree by a bootstrap analysis of 1,000 replications.

# Results

We sequenced 360 bp of the COI gene corresponding to locations of *Drosophila yakuba* Burla 1790–2150. This included 21 variable sites of which all were phylogenetically informative. Of the 14 populations sampled, we sequenced three unique haplotypes: GenBank accession nos: leaf mining EF190574 and external feeding EF190575 and EF190576). One haplotype is represented in all the leaf-mining populations. Two haplotypes are represented in the external-feeding populations. The population from Normal, IL, differs by one nucleotide from the rest of the external-feeding populations.

Our parsimony analysis of the sequences of the 14 populations of the ingroup and the sequence of the outgroup yielded a single most parsimonious tree with a length of 82 steps. The resulting tree is shown in Fig. 1 with the results of 1,000 bootstrap replicates included on the nodes. One hundred percent of the bootstrap replicates support the nodes of the parsimony tree. Other analyses were redundant given the clarity with which the two feeding types fell into the two monophyletic groups.

#### Discussion

What has been regarded as S. pilicornis (Smith 1979) was described several times in the literature (see synonymy under each species below). All of the early descriptions are based on the morphology of the adult. It is reasonable to presume that early taxonomists may have been observing the two different feeding types and perceiving morphological differences that we have overlooked until today. The size difference described by Rohwer (1909) is of particular interest. Rohwer (1909) described two new species, Schizocerus lineatus and Schizocerus collaris. In describing S. collaris, Rohwer (1909) writes, "Most closely related to S. lineatus, Roh, but is much smaller, and the lateral lobes of the mesonotum are dark brown, not reddish yellow [sic]." Given the significant size differences between the larvae and pupal cases of the two feeding types, it is possible that Rowher and others noticed a size dimorphism between the adults of the two types and correctly described two different species.

The two feeding types of *S. pilicornis* each form a monophyletic group and are clearly distinct lineages. Based on a divergence time of  $\approx 1$  million yr per 2% divergence (DeSalle et al. 1987), it seems that the two feeding types have been separate lineages for nearly 3 million yr. Further evidence of their genetic difference lies in the work of Gorske and Sell (1976). They examined four enzyme loci and found a Nei's D (Nei 1972) of 1.107. Thorpe (1982) suggests that 97% of populations that differ by greater than Nei's D = 0.16 represent different species. Despite the sympatric coexistence of the feeding types, it is clear that there has been little, if any, gene flow between them for some time.

The COI sequence divergence of 5.8%, we observe for the two feeding types of S. pilicornis is consistent with or exceeds COI sequence divergence of some closely related insect species. For example, in the Lepidoptera COI sequence divergence between two different species can be as low as 0.8% (Sperling et al. 1995), and in the dipteran genus *Drosophila* sibling species are usually divergent at the 1.5-2.7% level (Spicer 1995). A more appropriate comparison may be within the Hymenoptera. In the Hymenoptera, COI sequence divergence between closely related species is commonly <5% (Danforth et al. 1998, Danforth 1999). Beyond the sequence divergence, arguments for species status of the two feeding types are the allozyme work of Gorske and Sell (1976), size and color differences between the adults, and the difference in ecology represented by their distinct feeding behaviors. Consequently, we propose that the two lineages should be considered two separate species of Schizocerella.

### Recognition of Two Species

We recognize two species of "purslane sawflies," previously regarded as one color-variable species, both previously known as *Schizocerella pilicornis* (Holmgren) (Smith 1971, 1972, 1979, 1992). In addi-

tion to the previously reported genetic differences, presence of two feeding types (Gorske and Sell 1976, Gorske et al. 1977), and sequencing presented in this paper, several phenotypic differences were found to be constant. Study of voucher specimens known to be mining and external feeding collected and reared by E.F.C., study of specimens with data in the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM), and review of the literature support consistency of color and size characters that can be used to separate the two species. Names are available for both, and they are here regarded as Schizocerella pilicornis (Holmgren) (the leaf-mining type) and Schizocerella lineata (Rohwer) (the external-feeding type). Differences are based on females. Males of both are very similar, though size may be helpful in separating the species.

## Schizocerella pilicornis (Holmgren)

Schizocera pilicornis Holmgren 1868: 391.

Schizoceros lateralis Konow 1899: 403. (syn. by Malaise 1937).

Schizocerus zabriskei Webster and Mally 1900: 51. (syn. by Ross 1951).

Schizocerus sericeiformis Rohwer 1908: 112. (syn. by Ross 1937).

Schizocerus collaris Rohwer 1909: 13. (syn. by Ross 1937).

Schizocerella axillaris Forsius 1927: 19. (syn. by Benson 1963)

Schizocerella scapularis Forsius 1927: 19. (syn. by Benson 1963).

Diagnosis. Thorax of female with tegula, pronotum, and sometimes small lateral spot on each side of mesoprescutum orange, rest of thorax black. Lateral longitudinal band of abdomen narrow, mostly restricted to lateral, down-turned portions of terga and not extending onto dorsal surface. Size  $\approx 5-6$  mm in length.

Distribution. Specimens examined from Argentina (Salta, Buenos Aires); Mexico; Uruguay; United States (Arizona, California, Colorado, Connecticut, Washington, DC, Florida, Iowa, Indiana, Kansas, Maine, Maryland, Michigan, Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, Ohio, Oregon, Texas, Virginia, and Washington). It probably occurs throughout southern Canada, and the United States, south to Argentina. Specimens recorded from Bolivia, Brazil, and Guatemala and reported by Smith (1992) have not been reexamined.

Remarks. This also may be called the "black" form because of the mostly black mesonotum. All specimens in the USNM labeled as being leaf mining have the black mesonotum, and voucher specimens from Davis, CA, collected and reared by E.F.C. are this species.

Holmgren (1868) described *S. pilicornis* from "Brasilia (Rio Jan et Buen. Ayres)," and Konow (1899) described *S. lateralis* from "Argentina resp. et Uruguay." Holmgren's description stated that the thorax is black. Konow's description and types are those with

the black mesonotum. All other specimens examined from Argentina and Uruguay have the black mesonotum. Webster and Mally (1900) described the leafmining type *S. zabriskei* from Ohio, and all specimens examined from their study have the black mesonotum. Forsius (1927) described the black form *S. scapularis* (=*S. axillaris*) from Australia and described the mesonotum as black. The only specimen examined from Australia (NSW), is this black form. Other literature pertaining to this species is Dyar (1900), Markovitch (1916), Garlick (1922), and Frost (1925). Force (1965) treated a leaf-mining type from California, but the picture (fig. 1) seems to be *S. lateralis*.

S. sericeiformis and S. collaris, described from Nebraska and Colorado, respectively, have no associated biological data. Placement is based on study of type material and the original descriptions.

S. pilicornis was first described from South America in 1868 and is likely to be native there. Its presence in North America was not documented until 1900 by Webster and Mally. If it was common in North America before that time, certainly it would have been found and described by earlier workers.

## Schizocerella lineata (Rohwer), revised status

Schizocerus lineatus Rohwer 1909: 12. (as syn. of pilicornis by Ross 1937).

Schizocerus johnsoni MacGillivray 1909: 403. (as syn. of pilicornis by Ross 1937).

Diagnosis. Thorax of female with tegula, pronotum, lateral margins of mesoprescutum, and mesonotal lateral lobes orange to red. Lateral longitudinal stripe on abdomen broad, the orange extending onto dorsum, thus the black on dorsum appearing as a broad stripe the length of abdomen. Size larger than S. pilicornis, ≈7 mm in length.

Distribution. Specimens examined from Mexico; Salvador; United States (Alabama, Arizona, California, Connecticut, Washington, DC, Florida, Illinois, Indiana, Kansas, Louisiana, Maryland, Massachusetts, Michigan, Missouri, Mississippi, Nebraska, New Jersey, New Mexico, New York, Ohio, Oregon, Pennsylvania, Texas, Virginia, Wisconsin). Apparently widespread, probably in southern Canada, throughout the United States, and south to Central America. No species of *Schizocerella* occur in Europe; all are Western Hemisphere except for one introduced into Australia.

Remarks. This external-feeding species may be called the "red form" because of the mostly orange mesonotum with only a triangular patch on the prescutum and the scutellum black. Voucher specimens labeled as being external feeders collected and reared by EFC from Illinois are this form. Although there are many specimens in the USNM with host data, most give the host plant, *Portulaca*, but do not cite habits. Although this is commonly collected and widely distributed, little has been published on its biology. Its first occurrence is documented by the description of *S. lateralis* by Rohwer in 1909. It has not been found in South America where *S. pilicornis* occurs.

The descriptions of *S. lineatus* and *S. johnsoni* do not contain biological information. Interpretation is based on study of type material and the original descriptions. *Schizocerus lineatus* was described from Nebraska and *S. johnsoni* from New Jersey. Both were published the same year in the same journal, but *S. lineatus* takes precedence.

## Evolution of Feeding Habits in Schizocerella

Given that the two feeding types are distinct lineages, the question arises, which is the derived traitleaf mining or external feeding? Gorske and Sell (1976) suggested that the external feeding type of S. pilicornis is the derived lineage, presumably because of its recent discovery and what was then thought to be its narrow distribution. Although it is true that more recently derived lineages tend toward a more narrow distribution than ancestral lineages, the uniqueness of the leaf-mining trait of S. pilicornis within the family Argidae speaks a different story. Given that all other species in the family Argidae for which the feeding behavior is known are external feeding, it is most likely that the congeners of S. pilicornis are external feeding as well. The most parsimonious explanation for the two feeding types of S. pilicornis, therefore, is that the leaf-mining type is derived from the external-feeding type. Such a scenario requires only one shift in feeding behavior. If external feeding is the derived trait, the mining type of S. pilicornis would have evolved leaf mining from an external feeding ancestor and then given rise to the external feeding type, two evolutionary shifts. Also, both Pschorn-Walcher and Altenhofer (1989) and Connor and Taverner (1997) argue that leaf mining is a derived trait in the Hymenoptera in

A phylogenetic analysis of mitochondrial DNA has the potential to identify the polarity of this trait. However, the nature of the sequences from this study, two haplotypes representing the two feeding types, and the distantly related outgroup does not provide any information on which feeding behavior is the ancestral trait. To determine the ancestral trait with certainty it would be necessary to include more species of the genus *Schizocerella* and its close relatives and to know their feeding behaviors. The three other described members of the genus are all found in South America (Smith 1971, Smith 1992); however, none of their feeding behaviors are known.

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